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(54) Title: AGENTS THAT ACTIVATE OR INHIBIT TOLL-LIKE RECEPTOR 9

(57) Abstract: The present invention includes molecules that bind to a peptidic segment on TLR9 and mimic the effects of the CpG motif. The CpG mimicking agents include, but are not limited to, antibodies, small-molecule compounds, peptides, peptide mimetics, and nucleic acids, including compositions comprising molecules that bind to a peptidic segment on TLR9 and mimic the effects of the CpG motif suitable for administering to a patient in need of treatment, optionally in combination with, for example, an excipient, diluant, or carrier. In addition, the present invention includes those molecules which bind to the TLR9's CXXC motifs at ^{255}Cys-Arg-Arg ^{258}Cys (as CRRC) or at ^{265}Cys-Met-GIu ^{268}Cys (as CMEC). The present invention includes methods for modulating the immune response by inducing a Th1-type response comprising administering molecules that bind to TLR9 and mimic CpG function. These molecules also shift the host cellular response away from a Th2-type response toward the Th1-type response. Thus, administering the molecules of the present invention that bind to TLR9 may avoid the risk of Th2-mediated, immunization-induced anaphylaxis, making this method useful in immunotherapy and asthma treatment. The molecules of the present invention may be administered in combination with a particular allergen.

AGENTS THAT ACTIVATE OR INHIBIT TOLL-LIKE RECEPTOR 9

FIELD OF THE INVENTION

The present invention is related to the identification of an epitope on the toll-like receptor, TLR9, responsible for interaction with the CpG motif.

BACKGROUND

An organism's immunity includes all of the mechanisms it uses for protection against foreign environmental agents, *e.g.,* microorganisms, foods, chemicals, drugs, or pollens. In vertebrates, immunity may be either innate or acquired (adaptive).

Innate immunity is conferred by elements with which the organism is born. These include physical barriers, such as skin and mucosal membranes, internal elements, such as fever and coughs, a variety of chemical elements, such as interferon, serum proteins, *e.g.*, lysozyme and polyamines, and cellular elements, *e.g.,* macrophages, dendritic cells, and granulocytes.

It has been shown that certain bacterial DNA, but not vertebrate DNA, can activate an organism's innate immunity (Tokunaga, T. et al., *J. Natl. Cancer Institute* 72:955-962 (1984); Messina, J.P. et al., *J. Immunol* 147:1759-1764 (1991)). It is now becoming clear that innate immune cells, such as macrophages and dendritic cells, heavily influence the adaptive immune responses of T and B lymphocytes. Innate immune cells play an important role in antigen presentation, which determines whether T-cells respond at all and whether the induced T-cell response is predominantly a Th1 or Th2 response (Aderem, A. et al., *Nature* 406:782-787 (2000)). The immune response to any antigen can be either Th1 or Th2, and each induces different cytokines, antibodies, and cellular responses.

In studying this innate immune response to bacterial DNA, it was determined that the recognition of bacterial DNA as "foreign," and induction of an enhanced immune response against it, may be due to segments of unmethylated "CG" dinucleotide sequences in the DNA (designated "CpG" sequences). Unmethylated CpG dinucleotides are abundant in all bacterial genomes, as well as viral and invertebrate eukaryotic genomes (Bird, A.P., *Nuc. Acids Res.* 8:1499-1504 (1980); Burge, C., et al., *Proc. Nat. Acad. Sci.* USA 89:1358-1362 (1992)). In vertebrate genomes, CpG motifs can be found only at low frequency, and about 70% of vertebrate CpG motifs found are methylated. *Id.* Only about one-quarter of the predicted number of CG sequences are present in vertebrates as would be expected if base frequency were random (Bird, A.P., *Trends in Genetics* 3:342-347 (1987)). In contrast to bacterial CGs, human CG sequences are most frequently preceded by a C or followed by a G. These human CG sequences either have no effect on the immune system or may even mediate inhibition of an immune response. (Krieg, A.M. et al., *Proc. Natl. Acad. Sci.* USA 95:12631-12636 (1998)).

Bacterial CpG-DNA is a potent Th1-like adjuvant triggering a strong Th1 biased antibody response, and concomitantly suppressing the Th2 response (Davis, H.L. et al., *J. Immunol.*160:870-876 (1998)). CpG-DNA is also a potent single B-cell mitogen which is capable of driving more than 95% of B-cells into an activated state (Krieg, A.M. et al., *Nature* 374:546-549 (1995)). These innate immune responses can be mimicked by synthesized unmethylated CpG-containing oligodeoxynucleotides (CpG-ODNs). B-cells activated by bacterial CpG sequences or CpG-ODNs show increased expression of surface class-II major histocompatibility complex (MHC) molecules and the co-stimulatory

molecules B7-1 and B7-2 (Krieg, A.M., In: Delivery Strategies for Antisense Oligonucleotide Therapeutics, Ed. Akhtar, S., CRC Press, Inc., pp 177-190; Davis, H.L. et al., *J. Immunol.* 160: 870-876 (1998)). This suggests the possibility that the CpG "motif," composed of CG dinucleotides, may directly enhance the antigen-presenting function of B-cells. Although the effects of the CpG motif on T-cells are less clear, highly purified T-cells that are stimulated through the T-cell receptor show synergistic proliferative responses to CpGs, indicating a mechanism through which CpGs could promote antigen-specific T-cell responses (Bendigs, S. et al., *Eur. J. Immunol.* 29:1209-1218 (1999)).

CpG-ODNs strongly stimulate NK lytic activity and IFN-γ production (Tokunaga, T et al., *J. Natl. Cancer Institute* 72:955-962 (1984); Yamamoto S., *J. Immunol.* 148:4072-4076 (1992)). Antigen-presenting cells, such as monocytes and dendritic cells, are activated by CpG-ODNs resulting in the production of Th1 cytokines (Jakob, T et al., *J. Immunol.* 161:3042-3049 (1998)), as well as MHC-class II molecules and co-stimulatory B7-1 and B7-2 molecules (Stacy, K.J. et al., *J. Immunol.* 157:2116-2122 (1996); Sparwasser, T. et al., *Eur. J. Immunol.* 28:2045-2054 (1998)).

CpG-ODNs are, therefore, potent inducers and stimulators of a wide range of antigen-dependent and antigen-independent immune responses, and can be used for developing vaccines against cancer and infectious diseases. Since CpG-ODNs activate NK cells, they are useful for enhancing the antibody dependent cellular cytotoxicity (ADCC) of anti-tumor antibodies. CpG-ODNs can shift the T-cell response from a Th2-type response to a Th1-type response, which can result in down-modulating of the allergic responses (Kline, J.N. et al., *J. Immunol.* 160:2555-2559 (1998); Sur, S. et al., *J. Immunol.* 162:5575-5582

(1999); Shirota, H. et al., *J. Immunol.* 164:5575-5582 (2000); Jahn-Schmid, B. et al., *J. Allergy Clin. Immunol.* 104:1015-1023 (1999); Broide, D.H. et al., *J. Clin. Immunol.* 21:175-182 (2001)).

Interestingly, the CpG-DNA driven innate immune response can be ablated by changing the CpG motif to a GC dinucleotide or by methylating the cytosine. This clear structure-function relationship implied the existence of a receptor with specificity for the unmethylated CpG motif. Recently, in a mouse gene-knockout study, it was shown that CpG function was completely dependent on the recently discovered toll-like receptor 9 (TLR9) (Hemmi, H. et al., *Nature* 408:740-745 (2000)). Based on *in vitro* studies, it is thought that the CpG motif interacts with a TLR9 after CpG-DNA has been internalized in endosomes (Wagner, H., *Immunity* 14:499-502 (2001)).

The TLR family consists of phylogenetically conserved transmembrane proteins that mediate innate immunity and are essential for microbial recognition. The extracellular region of these receptors contains multiple leucine-rich repeats (LRRs) and a carboxy-terminal cysteine-rich domain having homology to the cytoplasmic domain of IL1R. The first TLR, d*Toll,* was found in Drosophila, and plays an important role in innate immune responses to fungal infection (Anderson, K.V., *Curr. Opin. Immunol.* 12:13-19 (2000); Means, T.K., *Life Sci.* 68:241-258 (2000)). Other TLR members were subsequently found in other organisms. TLR2 mediates immune responses to peptidoglycan (PGN) and TLR4 mediates immune responses to lipopolysaccharides (LPS). Human TLR9 (PDB Accession Number: AAF78037, SEQ ID NO: 1) was recently cloned (Chuang, T-H et al., *Eur. Cytokine Netw.* 11:372-378 (2000)). The exact binding site of unmethylated CpG on TLR9 has not been previously defined.

Summary of the Invention

The present invention includes molecules that bind to a peptidic segment on TLR9 and mimic the effects of the CpG motif. The CpG mimicking agents include, but are not limited to, antibodies, small-molecule compounds, peptides, peptide mimetics, and nucleic acids. In addition, the invention includes compositions comprising molecules that bind to a peptidic segment on TLR9 and mimic the effects of the CpG motif suitable for administering to a patient in need of treatment, optionally in combination with, for example, an excipient, diluant, or carrier.

In addition, the present invention includes those molecules which bind to the TLR9's CXXC motifs at ^{255}Cys-Arg-Arg-^{258}Cys (as CRRC) or at ^{265}Cys-Met-Glu-^{268}Cys (as CMEC).

The present invention also includes methods of making molecules that bind to TLR9 and mimic the effects of the CpG motif. These methods include making monoclonal antibodies to the CpG epitope of TLR9.

In addition, the present invention includes a method of treating TLR9-mediated diseases comprising administering a CpG-mimicking agent including, but are not limited to, antibodies, small-molecule compounds, peptides, peptide mimetics, and nucleic acids. Nucleic acids include oligonucleotides. These molecules may be used to treat, prevent, or ameliorate disorders such as tumors, cancer, or pathogenic infections, such as those caused by viruses, fungi, bacteria, or parasites.

The present invention also includes compositions suitable for administering to a patient suffering from an allergic disease comprising molecules

that bind to TLR9 and mimic CpG function, optionally in combination with, for example, an excipient, diluant, or carrier.

The present invention includes a method of modulating the immune response by inducing a Th1-type response comprising administering molecules that bind to TLR9 and mimic CpG function. These molecules also shift the host cellular response away from a Th2-type response toward the Th1-type response. Thus, administering the molecules of the present invention that bind to TLR9 may avoid the risk of Th2-mediated, immunization-induced anaphylaxis, making this method useful in immunotherapy and asthma treatment. The molecules of the present invention may be administered in combination with a particular allergen.

In addition, the present invention includes administering molecules that bind to TLR9 and mimic CpG function as an artificial adjuvant in a mammal such as a mouse or a human. The present invention includes a method of vaccination in a subject by administering a vaccine antigen or an antigen encoded in a DNA vaccine and a molecule or composition that binds to a peptidic segment on TLR9 and mimics the effects of the CpG motif.

The present invention also includes immunogens comprising synthetic peptides, recombinant proteins or a DNA encoding the peptide or the recombinant protein derived from TLR9 containing CRRC and/or CMEC, which induce the production of antibodies that bind to the epitope of TLR9 responsible for binding CpG, particularly at CRRC and/or CMEC, and activate the receptor's functions. These immunogens can be administered to a subject in order to immunize against cancer or allergic reactions.

Another embodiment of the invention includes molecules that bind TLR9 and inhibit or antagonize the function of the receptor. The present invention includes molecules that antagonize the effects of CpG.

Another embodiment of the present invention includes gene constructs of antibodies or fragments thereof that bind to TLR9 for therapy. Upon introduction into a suitable host, the TLR9 antibody gene constructs will direct the synthesis of an antibody (or its fragments) capable of binding to TLR9 and either mimicking CpG function or inhibiting the function of the TLR9 receptor. The gene construct to be expressed may also encode the epitope of TLR9 responsible for interaction with CpG. These constructs include genes for whole antibody molecules as well as modified or derived forms thereof, including immunoglobulin fragments like Fab, single chain Fv (scFv) and $F(ab')_2$. The gene construct can be introduced into a host with conventional gene therapy techniques, including naked DNA, DNA incorporated in liposomes, DNA conjugated to lipids or to lipid derivatives, or via suitable plasmids or recombinant viral vectors.

Brief Description of the Figures

Figure 1 shows the human cDNA sequence of toll-like receptor 9.

Figure 2 shows the protein sequence for human toll-like receptor 9.

Detailed Description of the Invention

It was reported that unmethylated CpGs bind to a peptide motif CXXC (two cysteine residues flanking two amino acids) (Voo, K.S. et al., *Mol. Cell. Biol.* 20:2108-2121 (2000)). Based on this information, we examined the amino acid sequence of human TLR9. We identified two CXXC motifs at ^{255}Cys-Arg-Arg-^{258}Cys (as CRRC) and at ^{265}Cys-Met-Glu-^{268}Cys (as CMEC). We now propose that the peptide segment encompassing these two CXXC motifs is responsible

for CpG interaction with TLR9 and therefore is important for CpG-mediated functions. Therefore, the present invention is related to the identification of the epitope on TLR9, which is responsible for the interaction with the CpG motif.

This invention also covers the making and use molecules that bind to this peptidic segment on TLR9 and mimic the effects of CpG. The CpG-mimicking agents could be, but not limited to, antibodies, small-molecule compounds, peptides, peptide mimetics, and nucleic acids. These novel agents could be used in humans to treat cancer and infectious diseases, such as those caused by intracellular pathogens like *Leishmania, Listeria, Francisella, Schistosoma,* ebola, anthrax, and malaria. In addition, these molecules could be used to treat allergic diseases, such as but not limited to, allergic rhinitis and asthma. These novel agents could be used either alone or in combination or as conjugates when administered to humans.

Monoclonal antibodies to TLR9 that mimic CpG functions can be made by immunizing animals such as rodents, with synthetic peptides or recombinant proteins encompassing a peptidic segment derived from human TLR9 containing, *e.g.,* CRRC or CMEC or both. Alternatively, the immunogen could be DNA encoding the peptide segment derived from human TLR9. Antibody molecules of the present invention include polyclonal or monoclonal antibodies, single chain antibodies, as well as functional fragments thereof. Monoclonal antibodies include chimeric or humanized antibodies, human antibodies, or DeImmunised™ antibodies. Fragments of these antibodies include Fv, Fab, $F(ab')_2$, single or double chain Fv fragments which retain the antigen binding function of the parent antibody. The antibody may be produced by any recombinant method known in

the art and may be produced *in vitro* or *in vivo.* Single chain antibodies ("ScFv") and the method of their construction are described in U.S. Patent No. 4,946,778.

Techniques for producing antibodies follow:

Monoclonal Antibodies

Monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-

11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP2/0 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). The mouse myeloma cell line NS0 may also be used (European Collection of Cell Cultures, Salisbury, Wiltshire UK).

Culture medium in which hybridoma cells are grown is assayed for production of monoclonal antibodies directed against the antigen. The binding specificity of monoclonal antibodies produced by hybridoma cells may be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (Innis M. et al. In PCR Protocols. A Guide to Methods and Applications, Academic, San Diego, CA (1990), Sanger, F.S, et al. *Proc. Nat. Acad. Sci.* 74:5463-5467 (1977)). The hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant production of antibodies will be described in more detail below.

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., *Nature* 348:552-554 (1990). Clackson et al., *Nature* 352:624-628 (1991) and Marks et al., *J. Mol. Biol.* 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., *Bio/Technology* 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nuc. Acids. Res.* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; Morrison, et al., *Proc.*

Nat. Acad. Sci. USA 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically, such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

Another alternative is to use electrical fusion rather than chemical fusion to form hybridomas. This technique is well established. Instead of fusion, one can also transform a B-cell to make it immortal using, for example, an Epstein Barr Virus, or a transforming gene. (See, e.g., "Continuously Proliferating Human Cell Lines Synthesizing Antibody of Predetermined Specificity," Zurawaki, V. R. et al, in Monoclonal Antibodies, ed. by Kennett R. H. et al, Plenum Press, N.Y. 1980, pp 19-33.)

Hybridomas producing specific anti-TLR9 monoclonal antibodies may be identified by ELISA using TLR9-derived antigen and by cell binding assays using cells expressing human TLR9. The specific activity, either agonistic (mimicking CpG) or antagonistic (inhibiting CpG), of the antibodies will be tested by examining their effects on cell-surface molecule expression and Th1-type cytokine production in primary cultures of B-cells, T-cells, NK cells, monocytes/macrophages, and dendritic cells. The functionally interesting antibodies will further be tested in animal models, such as those for allergy and asthma, tumor, intracellular pathogenic diseases, and vaccines.

Humanized and Human Antibodies

A humanized antibody has one or more amino acid residues introduced into it from a source, which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-327 (1988); Verhoeyen, et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies have substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., *Proc. Natl. Acad. Sci.* USA, 89:4285 (1992); Presta et al., *J. Immunol.*, 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, the skilled researcher can produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. Such transgenic mice are available from Abgenix, Inc., Fremont, California, and Medarex, Inc., Annandale, New Jersey. It has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human

antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci.* USA 90:2551 (1993); Jakobovits et al., *Nature* 362:255-258 (1993); Bruggermann et al., *Year in Immunol.* 7:33 (1993); and Duchosal et al. *Nature* 355:258 (1992). Human antibodies can also be derived from phage-display libraries (Hoogenboom et al., *J. Mol. Biol.* 227:381 (1991); Marks et al., *J. Mol. Biol.* 222:581-597 (1991); Vaughan, et al., *Nature Biotech* 14:309 (1996)).

DeImmunised™ Antibodies

DeImmunised™ antibodies are antibodies in which the potential T cell epitopes have been eliminated, as described in International Patent Application PCT/GB98/01473. Therefore, immunogenicity in humans is expected to be eliminated or substantially reduced when they are applied *in vivo.*

Additionally, antibodies can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Preferred polymers, and methods to attach them to peptides, are shown in U.S. Pat. Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546 which are all hereby incorporated by reference in their entireties. Preferred polymers are polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has a preferred average molecular weight between 1000 and 40,000, more preferably between 2000 and 20,000, most preferably between 3,000 and 12,000.

Other Molecules

Other molecules suitable for use in the invention can be isolated or screened from compound libraries by conventional means. An automated system for generating and screening a compound library is described in U.S. Patent Nos. 5,901,069 and 5,463,564. A more focused approach involves three-

dimensional modeling of the binding site, and then making a family of molecules, which fit the model. These are then screened for those with optimal binding characteristics.

Gene Constructs

The gene constructs of the present invention may be incorporated into a viral genome and subsequently packaged into suitable viral particles, which allows for a highly efficient gene delivery through viral infection. Exemplary viral vectors commonly used for gene therapy include retrovirus vectors, adenovirus vectors, and adeno-associated virus (AAV) vectors. The more recently developed viral vectors suitable for genetic therapy include lentivirus (HIV-1 or HIV-2 based vectors), and alphavirus vectors (based on Sindbis virus and Semliki Forest virus). The gene constructs can be incorporated into viral genomes of retroviruses, lentiviruses or AAV vectors by subcloning of the transcriptional units into appropriate cassette vectors containing necessary sequences for virus packing. Upon DNA transfection of the resulting constructs into appropriate packaging cell lines that produce viral components, the recombinant viral genomes can be properly packaged into viable viral particles.

To incorporate the gene constructs into an adenoviral viral genome, an additional step is generally taken. Since the adenoviral genome is approximately 36 Kbp long, it is not convenient to directly insert the antibody gene into the genome through restriction endonuclease digestion and ligation. Instead, genes are inserted in a cassette vector such as pAvCvSv (Kobayashi K et al. (1996) *J. Biol. Chem.* 22:6852 - 60). The vector has a pBR322 backbone and contains adenovirus type 5 (Ad5) 5' inverted terminal repeats (ITR), the Ad5 origin of replication, the Ad5 encapsidation signal, the E1a enhancer, multiple cloning

sites, and Ad5 sequence from nucleotide positions 3328 to 6246, which serve as a homologous recombination fragment. The resulting plasmid is then co-transfected into an appropriate host cell line, such as 293 cells (Graham FL, et al., *J. Gen. Virol.* 36:59-72 (1977)), along with a DNA fragment containing the bulk of the adenoviral genome with deletions in certain vital regions, such as the E1 and E3 genes. Homologous recombination between two DNAs in overlapping regions would allow the generation of a recombinant viral genome harboring the anti-TLR9 genes. This recombinant genome will be subsequently packaged into viable infectious viral particles in the 293 host cells. Incorporation of genes into the genome of alphaviruses or other viruses with a large genome can be similarly carried out to generate recombinant virus.

These gene constructs can be prepared as plasmids, which can be delivered to host cells or tissues, either directly or as naked DNA, or as DNA incorporated in liposomes, conjugated with appropriate lipid components, or incorporated in viral vectors. They are preferably injected for administration. The gene constructs will be expected to direct the synthesis of molecules that bind to TLR9 or antibody fragments thereof, which will gradually enter the blood stream to interact with TLR9. The recombinant virus constructs can be administered into an individual with allergic diseases via intra-muscular, intravenous, or subcutaneous routes. The dosage can be determined by extrapolating from animal experiments or determined in human clinical trials.

In another embodiment, cells are transfected to express intrabodies that specifically target, bind to, or inhibit the TLR9 receptor. An "intrabody" as used herein is an antibody that is expressed and active inside a cell. Intrabodies are typically not secreted and instead are directed to intracellularly expressed targets.

The intrabodies typically bind to targets within the cell and thereby trap the targets in an intracellular compartment (e.g., the ER). Intrabodies are well known to those of skill in the art (see, e.g., Chen et al., *Hum. Gene Therap.* 7: 1515-1525 (1996); Marasco *Immunotech.* 1: 1-19 (1995); and Maciejewski et al. *Nature Med.* 1: 667-673 (1995)). In principle, the high affinity and selective binding properties of intrabodies can be used to modulate cellular physiology and metabolism by a wide variety of mechanisms. For example, binding of an intrabody may be used to block or stabilize macromolecular interactions, modulate enzyme function by occluding an active site, sequestering substrate or fixing the enzyme in an active or an inactive conformation as the need may be. Intrabodies may also be used to divert proteins from their usual cellular compartment for example by sequestering transcription factors in the cytoplasm, or by retention in the ER of the proteins destined for the cell surface. In this regard intrabodies may be useful in conjunction with the present invention to trigger signal transduction mimicking CpG.

Additional pharmaceutical vehicles could be used to control the duration of action of the molecules of the invention. They could be entrapped in microcapsules prepared by coacervation techniques or by interfacial polymerization (hydroxymethylcellulose or gelatin microcapsules) in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Methods of preparing liposome delivery systems are discussed in Gabizon et al., *Cancer Research* 42:4734 (1982); Cafiso, *Biochem Biophys Acta* 649:129(1981); and Szoka, *Ann Rev Biophys Eng* 9:467 (1980). Other drug delivery systems are known in the art and are described in, e.g., Poznansky et al., In: Drug Delivery

Systems (R. L. Juliano, ed., Oxford, N.Y. 1980), pp. 253-315; M. L. Poznansky, *Pharm Revs* 36:277 (1984).

Liquid pharmaceutical compositions may be lyophilized to prevent degradation and to preserve sterility. Methods for lyophilizing liquid compositions are known to those of ordinary skill in the art. Just prior to use, the composition may be reconstituted with a sterile diluent (Ringer's solution, distilled water, or sterile saline, for example), which may include additional ingredients. Upon reconstitution, the composition is administered to subjects.

The molecules of the present invention can be administered by any of a number of routes and are administered at a concentration that is therapeutically effective in the indication or for the purpose sought. To accomplish this goal, the antibodies may be formulated using a variety of acceptable excipients known in the art. Typically, the antibodies are administered by injection, either intravenously or intraperitoneally. Methods to accomplish this administration are known to those of ordinary skill in the art. It may also be possible to obtain compositions which may be topically or orally administered, or which may be capable of transmission across mucous membranes.

The dosage and mode of administration will depend on the individual and the agent to be administered. The dosage can be determined by routine experimentation in clinical trials or extrapolation from animal models in which the antibody was effective.

The foregoing description, terms, expressions, and examples are exemplary only and not limiting. The invention includes all equivalents of the foregoing embodiments, both known and unknown. The invention is limited only

by the claims that follow and not by any statement in any other portion of this document or in any other source.

Claims:

1. A molecule that binds to a peptidic segment on TLR9 and mimics the effects of the CpG motif.
2. The molecule of claim 1, which is an antibody or an immunologically functional fragment thereof, a peptide, oligonucleotide, peptidomimetic or an organic compound.
3. A composition comprising a molecule that binds to a peptidic segment on TLR9 and mimics the effects of the CpG motif.
4. The composition of claim 3, wherein the molecule is an antibody or an immunologically functional fragment thereof, a peptide, oligonucleotide, peptidomimetic or an organic compound.
5. The molecule of claim 1, wherein the molecule binds at least one of Cys-Arg-Arg-Cys or Cys-Met-Glu-Cys of TLR9.
6. An agonist anti-TLR9 molecule that binds to and stimulates TLR9.
7. The antibody of any one of claims 2, 4, or 6, wherein the antibody is a monoclonal antibody.
8. The monoclonal antibody of claim 7, wherein the antibody is chimeric, humanized, DeImmunised™ or human.
9. A cell line that produces the antibody of any one of claims 2, 4, or 6.
10. A cell line that produces the antibody of claim 9.
11. A method of treating a TLR-mediated disease comprising administering an effective amount of a molecule or composition that binds to a peptidic segment on TLR9 and mimics the effects of the CpG motif.

12. The method of claim 11, wherein the molecule is an antibody or an immunologically functional fragment thereof, a peptide, oligonucleotide, peptidomimetic or an organic compound.

13. The method of claim 12, wherein the molecule is an agonist anti-TLR9 molecule that binds to and stimulates TLR9.

14. The method of claim 12, wherein the molecule is a monoclonal antibody.

15. The method of claim 14, wherein the monoclonal antibody is chimeric, humanized, DeImmunised™ or human.

16. The method of claim 11, wherein the disease is an allergic disease.

17. The method of claim 11, wherein the disease is an infectious disease.

18. A method for enhancing the treatment of tumors or cancer, comprising administering a molecule or composition that binds to a peptidic segment on TLR9 in combination with an anti-tumor or anti-cancer agent, thereby enhancing the effect of the anti-tumor or anti-cancer agent.

19. A method of inducing a Th1-type response comprising administering an effective amount of a molecule or composition that binds to a peptidic segment on TLR9 and mimics the effects of the CpG motif.

20. A method for desensitizing a subject against the occurrence of an allergic reaction in response to contact with a particular allergen, comprising administering to the subject an effective amount of a molecule or composition that binds to a peptidic segment on TLR9 and mimics the effects of the CpG motif.

21. A method of immunizing a subject, comprising administering to the subject an antigen or an antigen encoded in a DNA vaccine and a molecule or

composition that binds to a peptidic segment on TLR9 and mimics the effects of the CpG motif.

22. An immunogen comprising a synthetic peptide, a recombinant protein, or a DNA encoding the peptide or the recombinant protein derived from TLR9 containing CRRC and/or CMEC, which induces production of antibodies which bind to the epitope of TLR9 responsible for binding CpG.

23. The immunogen of claim 22, wherein the molecule binds to CRRC and/or CMEC.

24. A method of immunizing a patient comprising administering a synthetic peptide, recombinant protein, or a DNA encoding the peptide or the recombinant protein derived from TLR9 containing CRRC and/or CMEC which induces production of antibodies which bind to the epitope of TLR9 responsible for binding CpG.

25. A molecule that binds to a peptidic segment on TLR9 and inhibits the function of the receptor.

26. A method of treating a TLR-mediated disease comprising administering an effective amount of a molecule or composition that binds to a peptidic segment on TLR9 and inhibits the function of the receptor.

27. A nucleic acid construct encoding an antibody or fragment thereof, which binds to a peptidic segment on TLR9 and mimics the effects of the CpG motif.

28. A recombinant expression vector comprising the nucleic acid of claim 27.

29. A cell transformed with the nucleic acid of claim 27.

30. A method of inducing a host cell to express a molecule that binds to a peptidic segment of TLR9 comprising administering a formulation including the nucleic acid of claim 27.

FIGURE 1 Nucleotide Sequence of toll-like receptor 9.

```
   1 ccacgcgtcc ggggacctcg agtgtgaagc atccttccct gtagctgctg tccagtctgc
  61 ccgccagacc ctctggagaa gcccctgccc cccagcatgg gtttctgccg cagcgccctg
 121 cacccgctgt ctctcctggt gcaggccatc atgctggcca tgaccctggc cctgggtacc
 181 ttgcctgcct tcctaccctg tgagctccag ccccacggcc tggtgaactg caactggctg
 241 ttcctgaagt ctgtgcccca cttctccatg gcagcacccc gtggcaatgt caccagcctt
 301 tccttgtcct ccaaccgcat ccaccacctc catgattctg actttgccca cctgcccagc
 361 ctgcggcatc tcaacctcaa gtggaactgc ccgccggttg gcctcagccc catgcacttc
 421 ccctgccaca tgaccatcga gcccagcacc ttcttggctg tgcccaccct ggaagagcta
 481 aacctgagct acaacaacat catgactgtg cctgcgctgc ccaaatccct catatccctg
 541 tccctcagcc ataccaacat cctgatgcta gactctgcca gcctcgccgg cctgcatgcc
 601 ctgcgcttcc tattcatgga cggcaactgt tattacaaga acccctgcag gcaggcactg
 661 gaggtggccc cgggtgccct ccttggcctg ggcaacctca cccacctgtc actcaagtac
 721 aacaacctca ctgtggtgcc ccgcaacctg ccttccagcc tggagtatct gctgttgtcc
 781 tacaaccgca tcgtcaaact ggcgcctgag gacctggcca atctgaccgc cctgcgtgtg
 841 ctcgatgtgg gcggaaattg ccgccgctgc gaccacgctc ccaacccctg catggagtgc
 901 cctcgtcact tcccccagct acatcccgat accttcagcc acctgagccg tcttgaaggc
 961 ctggtgttga aggacagttc tctctcctgg ctgaatgcca gttggttccg tgggctggga
1021 aacctccgag tgctggacct gagtgagaac ttcctctaca aatgcatcac taaaaccaag
1081 gccttccagg gcctaacaca gctgcgcaag cttaacctgt ccttcaatta ccaaaagagg
1141 gtgtcctttg cccacctgtc tctggccct tccttcggga gcctggtcgc cctgaaggag
1201 ctggacatgc acggcatctt cttccgctca ctcgatgaga ccacgctccg gccactggcc
1261 cgcctgccca tgctccagac tctgcgtctg cagatgaact tcatcaacca ggcccagctc
1321 ggcatcttca gggccttccc tggcctgcgc tacgtggacc tgtcggacaa ccgcatcagc
1381 ggagcttcgg agctgacagc caccatgggg gaggcagatg gaggggagaa ggtctggctg
1441 cagcctgggg accttgctcc ggccccagtg gacactccca gctctgaaga cttcaggccc
1501 aactgcagca ccctcaactt caccttggat ctgtcacgga acaacctggt gaccgtgcag
1561 ccggagatgt ttgcccagct ctcgcacctg cagtgcctgc gcctgagcca caactgcatc
1621 tcgcaggcag tcaatggctc ccagttcctg ccgctgaccg gtctgcaggt gctagacctg
1681 tcccacaata agctggacct ctaccacgag cactcattca cggagctacc acgactggag
1741 gccctggacc tcagctacaa cagccagccc tttggcatgc agggcgtggg ccacaacttc
1801 agcttcgtgg ctcacctgcg caccctgcgc cacctcagcc tggcccacaa caacatccac
1861 agccaagtgt cccagcagct ctgcagtacg tcgctgcggg ccctggactt cagcggcaat
1921 gcactgggcc atatgtgggc cgagggagac ctctatctgc acttcttcca aggcctgagc
1981 ggtttgatct ggctggactt gtcccagaac cgcctgcaca ccctcctgcc ccaaaccctg
2041 cgcaacctcc ccaagagcct acaggtgctg cgtctccgtg acaattacct ggccttcttt
2101 aagtggtgga gcctccactt cctgcccaaa ctggaagtcc tcgacctggc aggaaaccag
2161 ctgaaggccc tgaccaatgg cagcctgcct gctggcaccc ggctccggag gctggatgtc
2221 agctgcaaca gcatcagctt cgtggcccca ggcttctttt ccaaggccaa ggagctgcga
2281 gagctcaacc ttagcgccaa cgccctcaag acagtggacc actcctggtt tgggcccctg
2341 gcgagtgccc tgcaaatact agatgtaagc gccaaccctc tgcactgcgc ctgtggggcg
2401 gcctttatgg acttcctgct ggaggtgcag gctgccgtgc ccggtctgcc cagccgggtg
2461 aagtgtggca gtccgggcca gctccagggc ctcagcatct ttgcacagga cctgcgcctc
2521 tgcctggatg aggccctctc ctgggactgt ttcgccctct cgctgctggc tgtggctctg
2581 ggcctgggtg tgcccatgct gcatcacctc tgtggctggg acctctggta ctgcttccac
2641 ctgtgcctgg cctggcttcc ctggcggggg cggcaaagtg ggcgagatga ggatgccctg
2701 ccctacgatg ccttcgtggt cttcgacaaa acgcagagcg cagtggcaga ctgggtgtac
2761 aacgagcttc gggggcagct ggaggagtgc cgtgggcgct gggcactccg cctgtgcctg
2821 gaggaacgcg actggctgcc tggcaaaacc ctctttgaga acctgtgggc ctcggtctat
2881 ggcagccgca agacgctgtt tgtgctggcc cacacggacc gggtcagtgg tctcttgcgc
2941 gccagcttcc tgctggccca gcagcgcctg ctggaggacc gcaaggacgt cgtggtgctg
3001 gtgatcctga gccctgacgg ccgccgctcc cgctacgtgc ggctgcgcca gcgcctctgc
3061 cgccagagtg tcctcctctg gccccaccag cccagtggtc agcgcagctt ctgggcccag
3121 ctgggcatgg ccctgaccag ggacaaccac cacttctata accggaactt ctgccaggga
3181 cccacggccg aatagccgtg agccggaatc ctgcacggtg ccacctccac actcacctca
3241 cctctgcctg cctggtctga ccctcccctg ctcgcctccc tcaccccaca cctgacacag
3301 agcaggcact caataaatgc taccgaaggc taaaaaaaaa aaaaaaaaaa aaaaaaaaaa
3361 aaaaaaaaaa aaaaaaaaaa aaaa
```

FIGURE 2 Protein Sequence of Toll-Like Receptor 9

```
   1 mgfcrsalhp lsllvqaiml amtlalgtlp aflpcelqph glvncnwlfl ksvphfsmaa
  61 prgnvtslsl ssnrihhlhd sdfahlpslr hlnlkwncpp vglspmhfpc hmtiepstfl
 121 avptleelnl synnimtvpa lpkslislsl shtnilmlds aslaglhalr flfmdgncyy
 181 knpcrqalev apgallglgn lthlslkynn ltvvprnlps sleylllsyn rivklapedl
 241 anltalrvld vggncrrcdh apnpcmecpr hfpqlhpdtf shlsrleglv lkdsslswln
 301 aswfrglgnl rvldlsenfl ykcitktkaf qgltqlrkln lsfnyqkrvs fahlslapsf
 361 gslvalkeld mhgiffrsld ettlrplarl pmlqtlrlqm nfinqaqlgi frafpglryv
 421 dlsdnrisga seltatmgea dggekvwlqp gdlapapvdt pssedfrpnc stlnftldls
 481 rnnlvtvqpe mfaqlshlqc lrlshncisq avngsqflpl tglqvldlsh nkldlyhehs
 541 ftelprleal dlsynsqpfg mqgvghnfsf vahlrtlrhl slahnnihsq vsqqlcstsl
 601 raldfsgnal ghmwaegdly lhffqglsgl iwldlsqnrl htllpqtlrn lpkslqvlrl
 661 rdnylaffkw wslhflpkle vldlagnqlk altngslpag trlrrldvsc nsisfvapgf
 721 fskakelrel nlsanalktv dhswfgplas alqildvsan plhcacgaaf mdfllevqaa
 781 vpglpsrvkc gspgqlqgls ifaqdlrlcl dealswdcfa lsllavalgl gvpmlhhlcg
 841 wdlwycfhlc lawlpwrgrq sgrdedalpy dafvvfdktq savadwvyne lrgqleecrg
 901 rwalrlclee rdwlpgktlf enlwasvygs rktlfvlaht drvsgllras fllaqqrlle
 961 drkdvvvlvi lspdgrrsry vrlrqrlcrq svllwphqps gqrsfwaqlg maltrdnhhf
1021 ynrnfcqgpt ae
```

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/035695 A3

(54) Title: AGENTS THAT ACTIVATE OR INHIBIT TOLL-LIKE RECEPTOR 9

(57) Abstract: The present invention includes molecules that bind to a peptidic segment on TLR9 and mimic the effects of the CpG motif. The CpG mimicking agents include, but are not limited to, antibodies, small-molecule compounds, peptides, peptide mimetics, and nucleic acids, including compositions comprising molecules that bind to a peptidic segment on TLR9 and mimic the effects of the CpG motif suitable for administering to a patient in need of treatment, optionally in combination with, for example, an excipient, diluant, or carrier. In addition, the present invention includes those molecules which bind to the TLR9's CXXC motifs at ^{255}Cys-Arg-Arg ^{258}Cys (as CRRC) or at ^{265}Cys-Met-Glu ^{268}Cys (as CMEC). The present invention includes methods for modulating the immune response by inducing a Th1-type response comprising administering molecules that bind to TLR9 and mimic CpG function. These molecules also shift the host cellular response away from a Th2-type response toward the Th1-type response. Thus, administering the molecules of the present invention that bind to TLR9 may avoid the risk of Th2-mediated, immunization-induced anaphylaxis, making this method useful in immunotherapy and asthma treatment. The molecules of the present invention may be administered in combination with a particular allergen.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/23645

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/28 A61K39/39 A61K39/02 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, MEDLINE, EPO-Internal, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WAGNER H: "Bacterial CpG DNA activates immune cells to signal infectious danger." ADVANCES IN IMMUNOLOGY. UNITED STATES 1999, vol. 73, 1999, pages 329-368, XP009015569 ISSN: 0065-2776 the whole document ---	1-30
X	WAGNER HERMANN: "Toll meets bacterial CpG-DNA." IMMUNITY, vol. 14, no. 5, May 2001 (2001-05), pages 499-502, XP002250852 ISSN: 1074-7613 cited in the application page 500 -page 501 --- -/—	1-30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 September 2003	29/09/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Chakravarty, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KRIEG A M ET AL: "CpG motifs in bacterial DNA trigger direct B-cell activation." NATURE. ENGLAND 6 APR 1995, vol. 374, no. 6522, 6 April 1995 (1995-04-06), pages 546-549, XP002910391 ISSN: 0028-0836 cited in the application the whole document	1-30
X	HEMMI HIROAKI ET AL: "A Toll-like receptor recognizes bacterial DNA" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 408, no. 6813, 7 December 2000 (2000-12-07), pages 740-745, XP002168474 ISSN: 0028-0836 cited in the application the whole document	1-30
P,X	WO 01 55386 A (GLAXOSMITHKLINE ;RAY KEITH PAUL (GB); LEWIS ALAN PETER (GB)) 2 August 2001 (2001-08-02) claims 18-26	1-30
P,X	WO 02 06482 A (JAPAN SCIENCE & TECH CORP ;HEMMI HIROAKI (JP); AKIRA SHIZUO (JP)) 24 January 2002 (2002-01-24) claims 23-30	1-30
P,X	WO 01 93905 A (CISTEM BIOTECHNOLOGIES GMBH ;EGYED ALENA (AT); LINGNAU KAREN (AT);) 13 December 2001 (2001-12-13) the whole document	1-30

INTERNATIONAL SEARCH REPORT

[redacted]rnational application No.
PCT/US 02/23645

Box I Observations wher certain claims wer found unsearchable (Continuati n of item 1 of first sh et)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-30 relate to compounds and methods using them defined by reference to a desirable characteristic or property, namely the ability to agonise the TLR9 receptor (i.e. mimic natural ligand which consists of certain nucleic acids having the CpG motif).

The claims cover all compounds and methods having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any of such compounds or methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the products and methods by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. The search has been carried out for the concept of agonising the TLR9 recpetor. This is inspite of the fact that this subject-metter is neither sufficiently disclosed or supported in the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 02/23645

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0155386 A	02-08-2001	AU 2866501 A	07-08-2001
		EP 1250436 A1	23-10-2002
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		EP 1302541 A1	16-04-2003
		WO 0206482 A1	24-01-2002
		US 2003124655 A1	03-07-2003
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